

Motor and sensory re-innervation of the lung and heart after re-anastomosis of the cervical vagus nerve in rats

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There is no study in the literature dealing with re-innervation of the cardiopulmonary vagus nerve after its transection followed by re-anastomosis. In the present study, we explored the broncho-motor, heart rate and respiratory responses in rats at 2, 3 and 6 months after re-anastomosis of one cervical vagus trunk. The conduction velocity of A, B and C waves was calculated in the compound vagal action potential. We searched for afferent vagal activities in phase with pulmonary inflation to assess the persistence of pulmonary stretch receptor (PSR) discharge in re-innervated lungs. In each animal, data from the stimulation or recording of one re-anastomosed vagus nerve were compared with those obtained in the contra-lateral intact one. Two and three months after surgery, the conduction velocities of A and B waves decreased, but recovery of conduction velocity was complete at 6 months. By contrast, the conduction velocity of the C wave did not change until 6 months, when it was doubled. The PSR activity was present in 50% of re-anastomosed vagus nerves at 2 and 3 months and in 75% at 6 months. Respiratory inhibition evoked by vagal stimulation was significantly weaker from the re-anastomosed than intact nerve at 2 but not 3 months. Vagal stimulation did not elicit cardiac slowing or bronchoconstriction 6 months after re-anastomosis. Our study demonstrates the capacity of pulmonary vagal sensory neurones to regenerate after axotomy followed by re-anastomosis, and the failure of the vagal efferents to re-innervate both the lungs and heart.

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The vagus nerve plays a key role in the control of cardiac and respiratory function. Preganglionic vagal neurones in the brain stem drive postganglionic neurones via ganglia in the organ wall which mediate the vagal motor pathways to the sinus and atrio-ventricular nodes (Chuen-Wang *et al.* 1997) as well as the contractility of the tracheobronchial smooth muscle (Canning *et al.* 2002). Vagal sensory neurones have their cell bodies in nodose and jugular ganglia and project to nerve endings in the organ wall. Among the different pulmonary vagal receptors, the pulmonary stretch receptors (PSR), which detect the distension of the tracheobronchial wall at each inspiration, play a major role in the control of breath amplitude and duration (inspiratory 'off-switch' mechanism) during both eupnoea and exercise hyperventilation (Coleridge & Coleridge, 1986).

Nerve section followed by re-anastomosis leads to the successful sensorimotor reinnervation of skeletal muscles

(Decherchi *et al.* 2001; Hoh, 1975; Marqueste *et al.* 2004). However, we found no data in the literature with respect to organ re-innervation after re-anastomosis of the vagus nerve. Some human studies have evaluated the changes in motor or reflex cardiac and ventilatory control after a heart or heart–lung transplantation when the sectioned nerves were not sutured. They reported higher resting heart rates (Banner *et al.* 1988), impaired ventilatory and cardiac responses to exercise (Sciurba *et al.* 1988), a lack of arterial baroreflex modulation of the heart rate (Raczak *et al.* 1999), and an absence of bradycardic response to apnoea (Madden *et al.* 1997). A recent human study (Butler *et al.* 2001) has also shown that the respiratory sensations evoked by the injection of lobeline, known to activate the unmyelinated vagal afferents in healthy subjects, were absent in patients at 2 weeks after bilateral lung transplantation, but were partially restored within a year after transplantation.

Very few animal studies have explored the re-innervation of re-implanted lung and no attempt has been made to perform re-anastomosis of severed nerves. In one of two dogs subjected to a vagus nerve section, Edmunds *et al.* (1971) found a reappearance of the Hering–Breuer inflation reflex at 10 months after surgery, and of the deflation reflex at 16 months. In addition, they documented a bronchoconstriction in response to electrical vagal stimulation in the re-implanted lung. Lung transplantation studies in the rabbit (Eraslan *et al.* 1966) did not find any vagus nerve regeneration after re-implantation of the lung. By contrast, 8 months after lung transplantation in rats, Kawaguchi *et al.* (1998) identified sensory neurones in pulmonary isografts. On the other hand, studies focusing on the re-innervation of the gastrointestinal tract after a sole subdiaphragmatic vagal transection clearly indicated that after 45 weeks, successful regeneration of vagal afferents was present while this was not true for vagal efferents (Phillips *et al.* 2003). Thus, whether or not the vagus nerve re-anastomosis is followed by motor and sensory re-innervation remains to be demonstrated.

In the present study, we explored the recovery of cardiopulmonary vagal efferent and afferent function after re-anastomosis of one cervical vagus trunk. We focused on the bronchomotor and heart rate responses to vagal stimulation and on the respiratory inhibition elicited by vagal stimulation. We examined the vagal activity in phase with lung inflation in order to determine whether the PSR innervation was functionally re-established. Our data were compared with those obtained by stimulating or recording from the contra-lateral intact vagus nerve in each animal.

Methods

Animals

The experiments were conducted in 35 adult female Sprague–Dawley rats obtained from Iffa Credo (Les Oncins, France), weighing 278 ± 7 g (mean \pm s.e.m.) at inclusion in the study and, respectively, 401 ± 13 , 431 ± 18 and 513 ± 12 g at 2, 3 and 6 months after surgery. Housing, surgical procedures and assessment of analgesia were performed according to the French law on Animal Care Guidelines, and the protocol was approved by the Animal Care Committee of our University. Efforts were made to minimize the animal suffering and to only use the number of animals necessary to produce reliable scientific data.

The study comprised four groups of rats. In a control group ($n = 11$), no vagal surgery was performed to determine the normal values of nerve conduction velocities, and normal cardiac and respiratory responses to electrical vagal stimulation, and also to search for any dominant cardiopulmonary influences of the right

or left vagus. In the other three groups, the rats were randomly assigned to undergo a section of the right or left vagus nerve, immediately followed by re-anastomosis. Anaesthesia was repeated 2 months ($n = 8$), 3 months ($n = 8$) and 6 months ($n = 8$) after re-anastomosis.

General anaesthesia and monitoring

In all rats, general anaesthesia was performed with an intramuscular injection of ketamine (10 mg kg^{-1} , Sanofi, Libourne, France) followed by an intraperitoneal injection of pentobarbital sodium (Nembutal, 40 mg kg^{-1} , Sanofi). Throughout and after the operative procedure, the adequacy of the level of anaesthesia was judged from the changes in blood pressure and heart rate, and the absence of the corneal reflex and response to pain stimuli applied on the adipose pad of the animal's paw. The changes in circulatory variables and the re-appearance of reflex responses governed the injection of supplementary doses of pentobarbital sodium. A heating pad maintained the rectal temperature in the range of $37\text{--}38^\circ\text{C}$. A tracheotomy was performed and the animals were ventilated at constant volume (10 ml kg^{-1}) and frequency with a Harvard volumetric pump. The inhaled gas mixture contained 30% oxygen. A carotid artery was cannulated for retrograde measurement of arterial blood pressure using an electromanometer (Statham model P23 Db, Gould SA, Ballainvilliers, France).

Re-anastomosis of one cervical vagus nerve

One cervical vagus nerve was transected and its distal part was stitched with a minimal tension (Ethilon, 9/0; Omnium Medical, Neuilly, France) to the proximal part by two to three epineural sutures. The muscles and skin covering the cervical vagus nerve were closed with 3/0 sutures (Trinyl 3/0; Omnium Medical) and locally disinfected.

Post-operative management

The animals were housed in smooth-bottomed plastic cages at 22°C with a 12:12 h light–dark cycle. Food (Purina rat chow) and water were available *ad libitum*. Antibiotherapy was administered for the first 48 h post-operative period using intramuscular injections of cefotaxime (50 mg kg^{-1}). We repeated intrarectal administration of paracetamol (20 mg) for analgesia. Measurements of body weight were made daily during the first week then once a week.

Physiological measurements

No physiological measurements were performed at the time of nerve transection followed by re-anastomosis. Rats which underwent vagal re-anastomosis were

re-anaesthetized at the end of the 2, 3 or 6 month period. Then, as in control animals, recording of physiological variables and measurements of responses to vagal stimulation were performed. Three steel wire electrodes were implanted in the upper limbs and in the left hindlimb and connected to a neuroamplifier to record a standard electrocardiogram (ECG) lead. A pair of steel hooks was inserted in a diaphragmatic cupola through an abdominal incision. The diaphragmatic electromyogram (Edi) was amplified ($\times 10\,000$ – $30\,000$) and filtered (10 Hz to 3 kHz) by a neuroamplifier. The raw Edi was integrated using a leaky integrator with a time constant of 100 ms. The tracheal pressure (P_{Tr}) was measured from an electro-manometer (Statham model PM5) whose inlet was connected to a side arm of the tracheal cannula. All the variables (P_{Tr} , blood pressure, ECG, raw and integrated Edi) were displayed on a polygraph (Model TA 4000, Gould SA).

To record the afferent vagal activities, the whole distal nerve trunk (below the re-anastomosis when performed) was placed on a monopolar tungsten electrode. The nerve activity was referred to a nearby ground electrode, amplified ($\times 50\,000$ – $100\,000$) and filtered (10 Hz to 10 kHz) by a differential amplifier, and recorded on the polygraph.

Compound vagal action potentials were evoked with single pulses using a pair of platinum electrodes placed distally (or below the point of nerve repair) and connected to a neurostimulator through an isolation unit (Model S88, Grass Instruments, Quincy, Massachusetts, USA). A proximal tungsten electrode was placed rostral to the re-anastomosis, connected to a neuroamplifier ($\times 50\,000$ – $100\,000$) and filtered (10 Hz to 10 kHz). The vagal potentials were displayed on a storage oscilloscope (DSO 400, Gould SA) to average the compound action potentials evoked by the stimulation of the distal nerve. Pulse durations of stimuli were chosen to evoke the compound A and B waves (pulse duration, 0.1 ms) or the C wave (pulse duration, 1.0 ms), the three waves corresponding to heavy, thin and unmyelinated vagal fibres, respectively. We insured that the inversion of the stimulating and recording electrodes did not modify the data and also that the inter-electrode distance was always 1 cm. Nerve conduction velocities were calculated by dividing the inter-electrode distance by the conduction time of A, B and C waves which was measured by displacing the cursors on the oscilloscope screen from the pulse artefact to the peak of each wave.

In order to elicit a ventilatory and cardiac response to repetitive vagal stimulation, one pair of platinum electrodes was alternatively placed distally or cranially from the location of re-anastomosis. For each stimulation period, we insured that the inversion of the cathode and anode did not modify the response. As in our previous studies in dogs (Jammes *et al.* 1983) and rabbits

(Monier *et al.* 1995), we used a 1.0 ms pulse duration and different frequencies of stimulation in the range 10–100 Hz, searching for the stimulation frequency which elicited the highest apnoeic response and bradycardia and also a rise in P_{Tr} to assess bronchoconstriction. The maximal duration of stimulation period was only 20 s. In all the rats, the 50 Hz stimulation frequency was the most effective. In intact vagus nerves, the stimulation continued until the occurrence of a subsequent inspiratory activity. Unilateral vagal stimulation caused cardiac slowing but not cardiac arrest. For each stimulation bout, we measured the ventilatory response, the magnitude of P_{Tr} increase indicating bronchoconstriction, and the heart rate slowing. We used the respiratory inhibitory ratio, first proposed by Widdicombe (1961), defined as the ratio between the breath duration during pulmonary inflation and the mean duration of the 10 previous breaths. Previous studies in dogs (Jammes *et al.* 1983) and rabbits (Monier *et al.* 1995) have demonstrated that identical relationships exist between the respiratory inhibitory ratio and the changes in lung volumes or the frequency of electrical vagal stimulation. In our study, the time intervals were measured from Edi recordings. The quantification of the cardiac response to vagal stimulation was given by the ratio between the mean heart rate measured during stimulation and the first minute period preceding the stimulation which constituted the reference for each test (HRst/HRref).

After the final experiment, the rats were killed by an intravenous injection of a lethal dose of pentobarbital.

Statistical analysis

Values were expressed as mean \pm s.e.m. A Student's paired *t* test was used to compare left-to-right responses to electrical stimulation and recordings of afferent activities in control rats (intact nerves) and in rats studied 2, 3 and 6 months after surgery (intact *versus* re-anastomosed nerves). In operated rats, an analysis of variance followed by a *post hoc* Student–Neuman–Keuls test for all pair-wise comparisons was used to find any time dependence of the vagus nerve re-innervation throughout the 6 month recovery period.

A chi squared test was used to assess whether a significant difference existed between the number of bronchoconstrictor responses to vagal stimulation and the number of events during which phasic vagal afferent activity could be recorded in re-anastomosed and intact vagus nerves.

Results

Control data in intact vagus nerves

Figure 1 shows the conduction velocities in the three components of fibres in intact vagus nerves. No difference

was found between the right and left intact vagus nerves. In all control rats, as well as in non-operated vagus nerves in animals which underwent an unilateral re-anastomosis of the vagus nerve, the afferent vagal activity was modulated by pulmonary inflation (Fig. 2). The cardiopulmonary response to repetitive (50 Hz) unilateral stimulation of an intact vagus nerve always consisted of an increase in P_{Tr} (+40 to +80%), a respiratory inhibition and a cardiac slowing, as illustrated in Fig. 3 (left panel). In intact nerves, the statistical analysis did not reveal any significant difference in the respiratory and cardiac responses to right or left vagal stimulation (respiratory inhibitory ratio: right, 9.16 ± 2.0 ; left, 8.15 ± 1.80 ; HRst/HRref: right, $51 \pm 10\%$; left, $33 \pm 9\%$; number of tests = 43, 21 right and 22 left). The absence of any side-dominant respiratory and cardiac influences of one vagus nerve allowed us to pool right and left measurements in all rats.

Consequences of re-anastomosis of one vagus nerve

The changes in nerve conduction velocities are shown in Fig. 4. Compared with data obtained in intact nerves, we measured a transient reduction in conduction velocities of the A wave (2 and 3 months after surgery) and the B wave (only at 2 months) but complete recovery was obtained at 6 months. By contrast, the conduction velocity of the C wave was unaffected at 2 and 3 months but markedly increased at 6 months ($1.42 \pm 0.20 \text{ m s}^{-1}$ versus $0.60 \pm 0.06 \text{ m s}^{-1}$; $P < 0.05$).

While a phasic afferent vagal activity was recorded in all the intact vagus nerves, the afferent nerve response to periodic pulmonary inflation by the pump was only

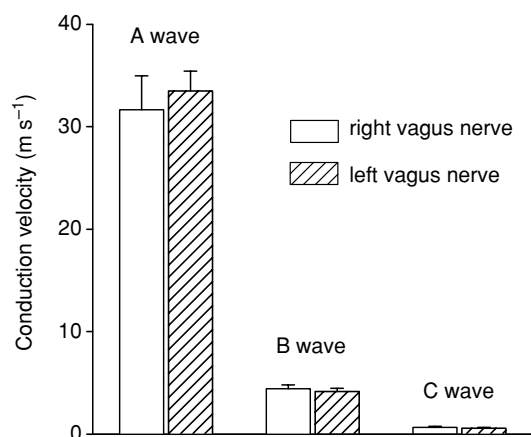


Figure 1. Conduction velocity in intact cervical vagus nerve measured in right and left nerves

For each side, we pooled data from control rats and contra-lateral non-operated nerves in rats studied 2, 3 and 6 months after surgery. The different waves in compound action potentials correspond to the three components (A, B and C waves), corresponding to heavy, thin and unmyelinated fibres, respectively. No right-to-left differences were noted.

present in 50% of the re-anastomosed vagus nerves at 2 and 3 months and in 75% at 6 months.

Figure 3 (right panel) shows that in re-anastomosed vagus nerve explored 6 months after the surgery, no change in P_{Tr} and HR occurred in response to vagal stimulation while the inhibitory respiratory response persisted. Compared with contra-lateral intact nerves, the respiratory inhibitory ratio was significantly reduced at 2 months but not at 3 and 6 months whereas the stimulation-induced cardiac slowing never reappeared (Fig. 5). In addition, the respiratory inhibitory ratio measured in control rats with both vagus nerves intact did not differ from that measured in operated rats when stimulating the uninjured vagus (8.4 ± 1.5 versus 8.6 ± 1.5 , respectively).

Discussion

We found no data in the literature on organ re-innervation after re-anastomosis of the vagus nerve whereas numerous reports concern spontaneous nerve regeneration, especially after organ transplantation. This rat study focused on the benefits of re-anastomosis of the cervical vagus nerve on respiratory and cardiac function. We observed that the bronchoconstrictor and bradycardic responses to vagal motor stimulation did not recover even 6 months after re-anastomosis of a cervical vagus nerve, whereas both PSR activity and stimulation-induced respiratory inhibition almost completely recovered. Compared with the intact vagus nerves, the conduction velocity measured in the myelinated vagal fibres (A and B waves of the compound action potential) 6 months after re-anastomosis was the same, whereas it was higher in the unmyelinated fibres (C wave).

To favour nerve repair we performed clean transection injuries of vagus nerves followed by immediate end-to-end epineurial anastomosis. Indeed, regenerating axones exhibit a preference to grow along the inside portion of remaining lamina tubes in the distal stump (Scherer & Eater, 1984) and, if the surgical nerve repair is

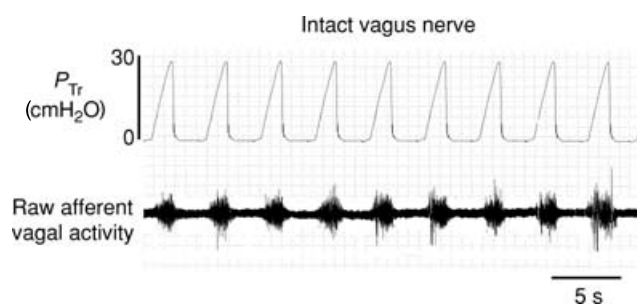


Figure 2

Example of recording of afferent nerve activities in phase with pulmonary inflation in an intact vagus nerve.

delayed, nerve regeneration can be markedly compromised (Giannini & Dyck, 1990).

The present data on reinnervation of the lungs and heart after re-anastomosis of the vagus nerve cannot be compared with those reported in human or animal recipients of heart–lung or lung transplants because, in available published data, vagal suture has never been performed after transplantation. Nevertheless, the human observations of a higher resting heart rate (Banner *et al.* 1988), a lack of arterial baroreflex modulation of the heart rate (Raczak *et al.* 1999) and an absence of bradycardic response to apnoea (Madden *et al.* 1997) suggest an absence of motor re-innervation of the transplanted heart, consistent with the present findings. The fact that an increased airway reactivity to inhaled methacholine was found in some patients receiving a heart–lung transplant (Banner *et al.* 1988) is insufficient to assess the status of vagal re-innervation. Indeed, by analogy with the parasympathetic denervation supersensitivity of the heart to infusion of methacholine (Dempsey & Cooper, 1968; Hageman *et al.* 1977; Chuen-Wang *et al.* 1997), we speculate that the increased airway response to cholinergic

agonists in transplanted lungs solely resulted from the changes in smooth muscle reactivity.

In the present rat study, the nerve conduction velocity in myelinated fibres (A and B waves) totally recovered within the 3 to 6 month period after re-anastomosis. Moreover, despite the reduction of the inhibitory respiratory response to vagal stimulation at 2 months after re-anastomosis, the response was not different at 3 months. Considering that the rate of vagal axonal regeneration is around 1 mm day^{-1} in adult rats (Kanje, 1991) and the length of vagal trunk between the brain stem and the pulmonary hilum was in the range 62–70 mm in our animals, the 3 month delay needed to recover a near-normal nerve conduction in re-anastomosed vagus may be considered as normal. Despite the expectation that regeneration rate should be the same in sensory and motor preganglionic vagal axones, we failed to measure any bronchomotor and heart rate responses to vagal stimulation, even 6 months after re-anastomosis. We hypothesized that during the rather long (3 months) period required for axonal regeneration in preganglionic neurones, the postganglionic vagal neurones in the airways and heart may have degenerated.

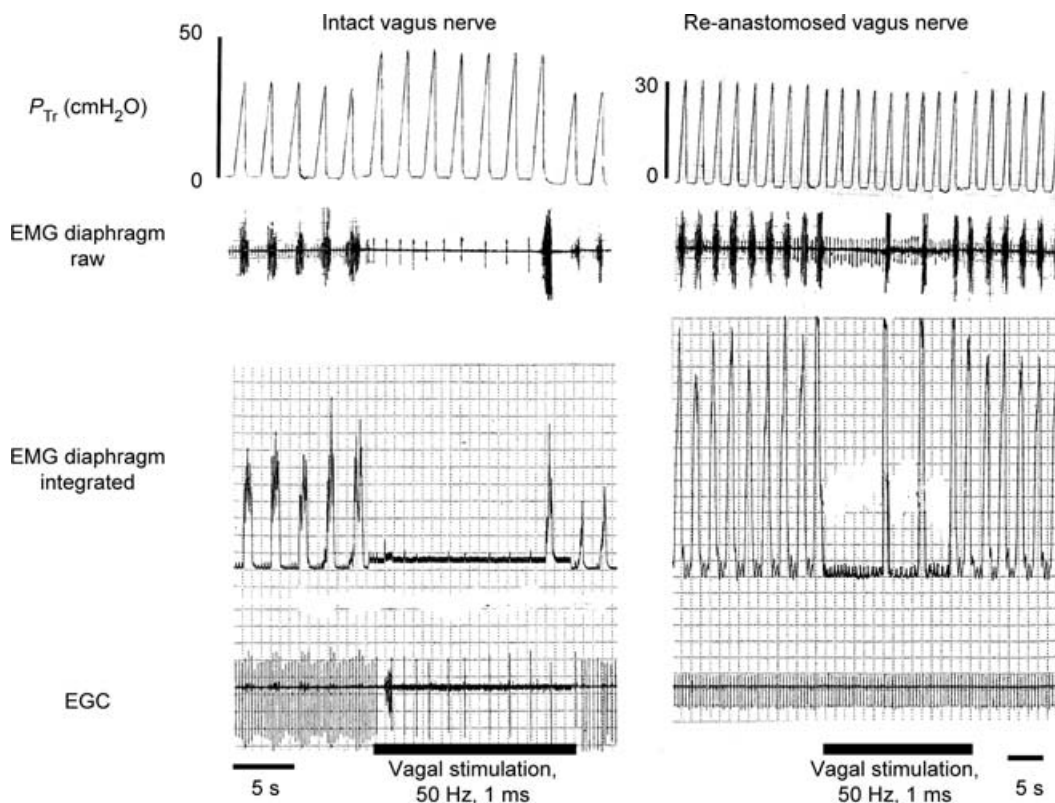


Figure 3. Examples of bronchomotor (P_{Tr} increase), respiratory (inspiratory inhibition) and cardiac (bradycardia) responses to repetitive (50 Hz) stimulation

Compared with an intact cervical vagus nerve (left panel), in the contralateral re-anastomosed cervical vagus nerve (right panel) explored 6 months after surgery we noted an absence of both the bronchomotor and cardiac responses to vagal stimulation whereas the ventilatory response reappeared. P_{Tr} , tracheal pressure; EMG, electromyogram; ECG, electrocardiogram.

Indeed, these neurones would be deprived of neurotransmitters as well as trophic factors normally released from the degenerated preganglionic neurones. There is recent evidence that airway-related vagal preganglionic neurones express brain-derived neurotrophic factors involved in neuronal plasticity (Zaidi *et al.* 2005). Our data for thoracic viscera are entirely consistent with those for the abdominal vagus which innervates the gut. Forty-five weeks after subdiaphragmatic vagotomies in rats, Phillips *et al.* (2003) labelled vagal afferents with germ agglutinin–horseradish peroxidase and efferents with cholera toxin subunit B–horseradish peroxidase. As in our study in the respiratory apparatus, Phillips *et al.* (2003) showed that the sensory re-innervation of the gut was present whereas motor fibres had failed to re-innervate the gastrointestinal tract.

The present data in re-anastomosed vagus are also consistent with our previous observations in the peroneal nerve (Decherchi *et al.* 2001) showing that a

re-anastomosed peripheral nerve has a higher conduction velocity in the group IV (unmyelinated) fibres than intact nerves and the response of mechanosensitive muscle endings to tendon vibration is preserved. Measurement of higher conduction velocities in group IV muscle afferents (Decherchi *et al.* 2001) and C vagal fibres (present study) may signify that we did not fully explore the response of unmyelinated fibres, but rather only recorded from a subgroup of regenerating thin myelinated axones. Indeed, the regeneration of peripheral nerves appears to require Schwann cells and endoneurial fibroblasts as well as the extracellular matrix produced by them (Sanes, 1989). Moreover, in peripheral nerve grafts there is an increase in the number of sprouts supported by myelinated fibres and a decrease of unmyelinated fibres (Decherchi *et al.* 1996).

The presence of an uninjured vagus nerve may have helped or hindered the recovery process of reflex respiratory responses to stimulation of the contralateral re-anastomosed vagus. Indeed, primary vagal afferent fibres from the lungs project on commissural subnucleus of the nucleus of the solitary tract (NTS), crossing the midline and synapsing in the contralateral nuclei of the

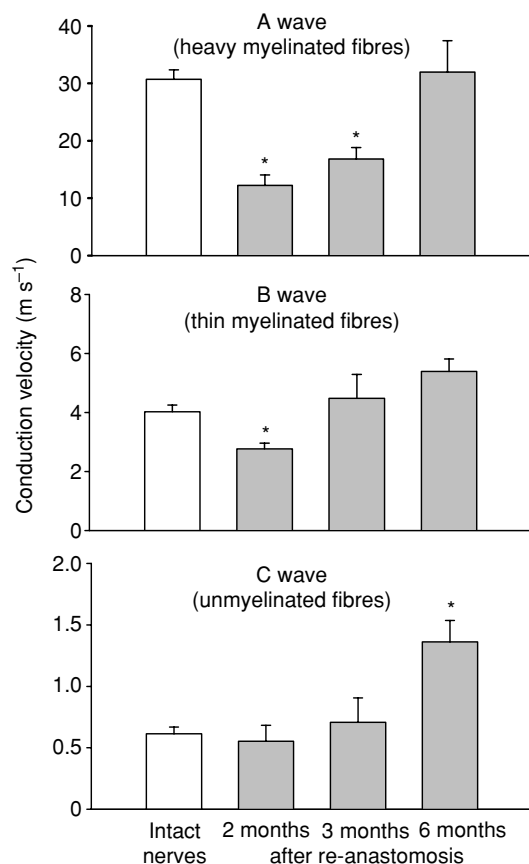


Figure 4. Conduction velocity of A, B and C waves of the compound vagal potential evoked by peripheral nerve stimulation in intact and re-anastomosed vagus nerves

Compared with intact vagus nerves, a significantly lower conduction velocity was measured in heavy myelinated vagal fibres (A wave) at 2 and 3 months and in thin myelinated fibres (B wave) at 2 months. By contrast, in myelinated fibres (C wave) the conduction velocity was significantly higher at 6 months. *Significant at the 0.05 level.

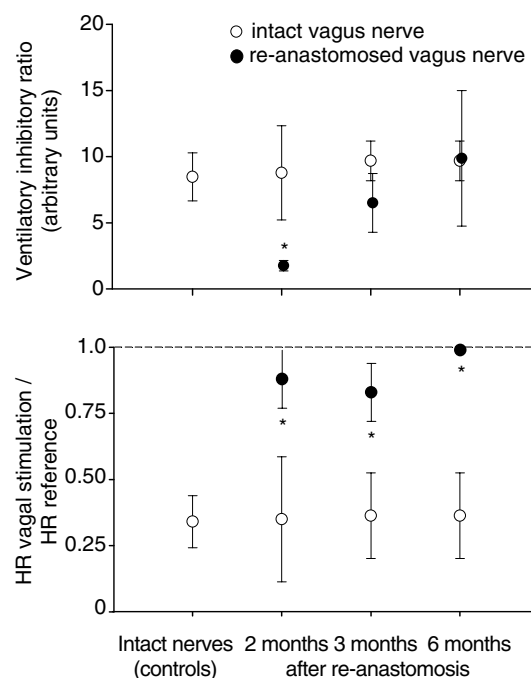


Figure 5. Ventilatory and cardiac responses to repetitive (50 Hz) stimulation of one intact or re-anastomosed cervical vagus nerve

In intact nerves in the control group as well as in contra-lateral intact nerves in rats which underwent nerve transection, the responses were not time dependent. In re-anastomosed nerves, the ventilatory response was significantly depressed at 2 months but not at 3 and 6 months. The heart rate response never recovered in re-anastomosed vagus nerves. Asterisk indicates a significant ($P < 0.05$) difference compared with the intact nerves.

NTS (Otake *et al.* 1992). Thus, it may be supposed that the reflex response to the stimulation of a single intact vagus nerve could be different to that measured in a preparation with both vagus nerves intact. However, compared with controls, we did not find any significant difference in the respiratory inhibitory ratio elicited by the stimulation of intact vagus nerve in rats which underwent a contra-lateral vagal re-anastomosis. In the de-afferented side, surviving nerve terminal fibres may continue to innervate target cells in the NTS, inhibiting their further re-afferentation after anastomosis and delaying the re-appearance of reflex responses. This hypothesis cannot be verified in our study but, even if this central inhibitory process had occurred, it cannot explain the disappearance of vagal afferent activities in phase with pulmonary inflation during the recovery period of re-anastomosis. Thus, the suppression of respiratory reflexes during regeneration of vagal fibres was also associated with the cessation of sensory pathways from the lungs.

It is difficult to understand how the sensory protoneurons carrying information from specialized mechanosensory nerve endings can find their way again after re-anastomosis following a complete nerve section. Some explanation may be given by data on peripheral nerve plasticity. It is well known that following an injury of a peripheral muscle nerve, the proximal stump (growing tip) of the regenerating nerve becomes mechanically sensitive (Tinel, 1919) and also that myelinated afferents show a spontaneous impulse activity (Johnson & Munson, 1991). These observations may also hold for the vagus nerve and could explain our data that PSRs continued to display afferent discharges in phase with the pulmonary inflation. Moreover, in skeletal muscles the maintenance of the activity and accompanying stimulation of the sensory muscle endings (Marqueste *et al.* 2004) improved the mechanosensory re-innervation after re-anastomosis. The persistence of ventilatory motion, eliciting periodic stimulation of vagal PSRs, may favour their regeneration.

The unmyelinated fibres represent the majority of the vagal motor fibres in rabbits (Evans & Murray, 1954) and cats (Agostoni *et al.* 1957; Jammes *et al.* 1982) as well as the vagal afferent axones in the feline lung (Jammes *et al.* 1982). The respective role played by myelinated and unmyelinated motor vagal fibres in the bronchoconstrictor response to vagal stimulation depends on the species. In rabbits, the selective stimulation of the sole myelinated axones (A and B fibres) or of the group C fibres induces bronchoconstriction (Lama *et al.* 1988). We found no similar data in rats but it was tempting to speculate that unmyelinated vagal motor fibres also play a role in these species. Since both the myelinated and unmyelinated preganglionic vagal motor fibres are connected with postganglionic neurones in the airways and heart, we suppose that the absence of any motor response to vagal stimulation of re-anastomosed vagi may signify that degeneration indifferently occurs in

neurones connected with either myelinated or non-myelinated fibres. In the present study we did not selectively explore the regeneration of unmyelinated afferent vagal fibres because we did not test their response to pulmonary deflation or inhalation of capsaicin. In all studied mammalian species, the group C bronchopulmonary afferents play a key role in the sensory innervation because they are polymodal receptors activated by both mechanical and chemical stimuli (Delpierre *et al.* 1981; Coleridge & Coleridge, 1986) and their tonic discharge controls the bronchoconstrictor vagal command (Jammes & Mei, 1979). Considering the present observations of an abnormally high conduction velocity of the C wave in the compound vagal potential after re-anastomosis, and also previous data on reduced response of the group IV (unmyelinated) muscle afferents to chemical stimulation after re-anastomosis of a muscle nerve (Decherchi *et al.* 2001), we may suspect an altered respiratory control by the bronchopulmonary vagal fibres in re-innervated lungs. This deserves further investigations.

This experimental animal study strongly suggests that immediate re-anastomosis of a sectioned vagus nerve leads to an almost complete recovery of the afferent pathways controlling the respiration, and also probably of the sensory information from the heart. This suggests that surgeons should consider the anastomosis procedure in order to enhance the potential for afferent nerve regeneration.

References

- Agostoni E, Chinnock JE, De Burgh Daly M & Murray JG (1957). Functional and histological studies of the vagus nerve and its branches to the heart, lungs, and abdominal viscera in the cat. *J Physiol* **135**, 182–205.
- Banner N, Guz A, Heaton R, Innes JA, Murphy K & Yacoub M (1988). Ventilatory and circulatory responses at the onset of exercise in man following heart or heart–lung transplantation. *J Physiol* **399**, 437–449.
- Butler JE, Anand A, Crawford MR, Glanville AR, McKenzie DK, Paintal AS, Taylor JL & Gandeia SC (2001). Changes in respiratory sensations induced by lobeline after human bilateral lung transplantation. *J Physiol* **534**, 583–593.
- Canning BJ, Reynolds SM, Anukwu LU, Kajekar R & Myers AC (2002). Endogenous neurokinins facilitate synaptic transmission in guinea pig airway parasympathetic ganglia. *Am J Physiol Regul Integr Comp Physiol* **283**, R320–R330.
- Chuen-Wang C, Eble JN & Zipes DP (1997). Efferent vagal innervation of the canine atria and sinus and atrioventricular nodes. *Circulation* **95**, 2573–2590.
- Coleridge HM & Coleridge JCG (1986). Reflexes evoked from tracheobronchial tree and lungs. In *Handbook of Physiology*, section 3, *The Respiratory System*, vol II, *Control of Breathing*, ed. Fishman AP, Cherniack NS, Widdicombe JG, Geiger SR, pp. 431–448. American Physiological Society Bethesda, MA.

- Decherchi P, Lammari-Barreault N & Gauthier P (1996). Regeneration of respiratory pathway within spinal peripheral nerve grafts. *Exp Neurol* **137**, 1–14.
- Decherchi P, Vuillon-Cacciottolo G, Darques LJ & Jammes Y (2001). Changes in afferent activities from tibialis anterior muscle after nerve repair by re-anastomosis. *Muscle Nerve* **24**, 59–68.
- Delpierre S, Grimaud C, Jammes Y & Mei N (1981). Changes in activity of vagal bronchopulmonary C fibres by chemical and physical stimuli in the cat. *J Physiol* **316**, 61–74.
- Dempsey PJ & Cooper T (1968). Supersensitivity of the chronically denervated feline heart. *Am J Physiol* **215**, 1245–1249.
- Edmunds LH, Graf PD & Nadel JA (1971). Reinnervation of the reimplanted canine lung. *J Appl Physiol* **31**, 722–727.
- Eraslan S, Hardy JD & Elliott RL (1966). Lung reimplantation. *J Surg Res* **5**, 383–388.
- Evans DHL & Murray JG (1954). Histological and functional studies on the fibre composition of the vagus nerve of the rabbit. *J Anat* **88**, 320–337.
- Giannini C & Dyck J (1990). The fate of Schwann cell basement membranes in permanently transected nerves. *J Neuropathol Exp Neurol* **49**, 550–553.
- Hageman GRF, Urthaler F & James TN (1977). Differential sensitivity to neuro-transmitters in denervated canine sinus node. *Am J Physiol Heart Circ Physiol* **233**, H211–H216.
- Hoh JF (1975). Selective and non-selective reinnervation of fast-twitch and slow-twitch rat skeletal muscle. *J Physiol* **251**, 791–801.
- Jammes Y, Bye PTP, Pardy RL & Roussos C (1983). Vagal feedback with expiratory threshold load under extracorporeal circulation. *J Appl Physiol* **55**, 316–322.
- Jammes Y, Fornaris E, Mei N & Barrat E (1982). Afferent and efferent components of the bronchial vagal branches in cats. *J Auto Nerv Sys* **5**, 165–176.
- Jammes Y & Mei N (1979). Assessment of the pulmonary origin of bronchoconstrictor vagal tone. *J Physiol* **291**, 305–316.
- Johnson RD & Munson JB (1991). Regenerating sprouts of axotomized cat muscle afferents express characteristics firing patterns to mechanical stimulation. *J Neurophysiol* **66**, 2155–2158.
- Kanje M (1991). Survival and regeneration of the adult rat vagus nerve in culture. *Brain Res* **550**, 340–342.
- Kawaguchi AT, Shirai M, Yamano M, Ishibashi-Ueda H, Yamatodani A & Kawashima H (1998). Afferent reinnervation after lung transplantation in the rat. *J Heart Lung Transplant* **17**, 341–348.
- Lama A, Delpierre S & Jammes Y (1988). The effects of electrical stimulation of myelinated and non-myelinated vagal motor fibres on airway tone in the rabbit and the cat. *Respir Physiol* **74**, 265–274.
- Madden BP, Shenoy V, Dalrymple-Hay M, Griffiths T, Millard J, Backhouse L, Clarke L & Murday A (1997). Absence of bradycardic response to apnea and hypoxia in heart transplant recipients with obstructive sleep apnea. *J Heart Lung Transplant* **16**, 394–397.
- Marqueste T, Alliez JR, Alluin O, Jammes Y & Decherchi P (2004). Neuromuscular rehabilitation by treadmill running or electrical stimulation after peripheral nerve injury and repair. *J Appl Physiol* **96**, 1988–1995.
- Monier A, Burnet H & Jammes Y (1995). Hypoxemia does not affect the strength of the inspiration-inhibiting Breuer-Hering reflex. *Neurosci Lett* **197**, 129–132.
- Otake K, Ezure K, Lipski J & Wong She RB (1992). Projections from the commissural subnucleus of the nucleus of the solitary tract: an anterograde study in the cat. *J Comp Neurol* **324**, 365–378.
- Phillips RJ, Baronowsky EA & Powley TL (2003). Long-term regeneration of abdominal vagus: efferents fail while afferents succeed. *J Comp Neurol* **455**, 222–237.
- Raczak G, Rovere MT, Mortara A, Assandri J, Prpa A, Pinna GD, Maestri R, D'Armin AM, Vigano M & Corbelli F (1999). Arterial baroreflex modulation of heart rate in patients early after heart transplantation: lack of parasympathetic reinnervation. *J Heart Lung Transplant* **18**, 399–406.
- Sanes J (1989). Extracellular matrix molecules that influence neural development. *Ann Rev Neurosci* **5**, 2415–2423.
- Scherer SS & Eater SS (1984). Degenerative and regenerative changes in the trochlear nerve of goldfish. *J Neurocytol* **13**, 519–565.
- Sciurba FC, Owens GR, Sanders MH, Griffith BP, Hardesty RL, Paradis IL & Costantino JP (1988). Evidence of an altered pattern of breathing during exercise in recipients of heart-lung transplants. *N Engl J Med* **319**, 1186–1192.
- Tinel J (1919). Les paresthésies précoces après suture ou greffe nerveuse. *Rev Neurol Paris* **26**, 521–526.
- Widdicombe JG (1961). Respiratory reflexes in man and other mammalian species. *Clin Sci* **109**, 712–722.
- Zaidi SI, Jaffri A, Doggett T & Haxhiu MA (2005). Airway-related vagal preganglionic neurones express brain-derived neurotrophic factor and TrkB receptors: implications for neuronal plasticity. *Brain Res* **1044**, 133–143.